Original Article

Azelnidipine Attenuates Cardiovascular and Sympathetic Responses to Air-Jet Stress in Genetically Hypertensive Rats

Minori NAKAMOTO[1)], Yusuke OHYA[1)], Atsushi SAKIMA[1)], Masanobu YAMAZATO[1)], and Shuichi TAKISHITA[1)]

Azelnidipine is a new dihydropyridine calcium channel blocker that causes minimal stimulation of the sympathetic nervous system despite its significant depressor effect. In the present study, we examined the effects of oral or intravenous administration of azelnidipine on cardiovascular and renal sympathetic nerve activity (RSNA) responses to air-jet stress in conscious, unrestrained stroke-prone spontaneously hypertensive rats. Oral administration of high-dose azelnidipine (10 mg/kg per day) or nicardipine (150 mg/kg per day) for 10 days caused a significant and comparable decrease in blood pressure, but low-dose azelnidipine (3 mg/kg per day) did not. Air-jet stress increased mean arterial pressure (MAP), heart rate (HR) and RSNA. High-dose azelnidipine significantly attenuated the increases in MAP, HR and RSNA in response to air-jet stress while nicardipine did not. Low-dose azelnidipine significantly attenuated the pressor response with a trend of decrease in RSNA. Intravenous injection of azelnidipine induced a slowly developing depressor effect. To obtain a similar time course of decrease in MAP by azelnidipine, nicardipine was continuously infused at adjusted doses. Both drugs increased HR and RSNA significantly, while the change in RSNA was smaller in the azelnidipine group. In addition, intravenous administration of azelnidipine attenuated the responses of MAP, HR, and RSNA to air-jet stress; by comparison, the inhibitory actions of nicardipine were weak. In conclusion, oral or intravenous administration of azelnidipine inhibited cardiovascular and sympathetic responses to air-jet stress. This action of azelnidipine may be mediated at least in part by the inhibition of the sympathetic nervous system. (*Hypertens Res* 2007; 30: 359–366)

***Key Words*: azelnidipine, air-jet stress, renal sympathetic nerve activity**

Introduction

Long-acting calcium channel blockers are widely used for the treatment of hypertension as a first-line drug. In contrast, short-acting calcium channel blockers are not recommended, because they sometimes cause reflex tachycardia through activation of the sympathetic nervous system together with stimulation of the renin-angiotensin system due to an excess and rapid decrease in blood pressure (*1*). The mechanism that explains the difference, in clinical trials, between the improvement of cardiovascular outcomes by short-acting calcium channel blockers and that by long-acting calcium channel blockers, is considered to be related to alterations of activation of the sympathetic nervous system (*2–4*). Namely, short-acting calcium channel blockers stimulate sympathetic activity while long-acting calcium channel blockers effect it only minimally.

The sympathetic nervous system has an important role in the regulation of cardiovascular homeostasis. Evidence suggests that hyperactivity of the sympathetic nervous system contributes to the pathophysiology of hypertension and tar-

From the [1)]Department of Cardiovascular Medicine, Nephrology and Neurology, School of Medicine, University of the Ryukyus, Okinawa, Japan.
Address for Reprints: Yusuke Ohya, M.D., Department of Cardiovascular Medicine, Nephrology and Neurology, School of Medicine, University of the Ryukyus, 207 Uehara, Nishihara-cho, Okinawa 903–0215, Japan. E-mail: ohya@med.u-ryukyu.ac.jp
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get-organ damage (*5*–*7*). It has also been demonstrated that patients with hypertension and experimental hypertensive animals exhibit an exaggerated sympathetic activity and cardiovascular response to various stresses, including mental and environmental stress, compared with their normotensive counterparts (*8*, *9*). Although it has not been fully clarified whether an exaggerated response to stress is related to the development of hypertension and cardiovascular disease, several studies have suggested an independent and positive relationship between the magnitude of blood pressure variability and target-organ damages, such as a left ventricular mass, early carotid atherosclerosis, or a subcortical brain lesion in normotensive or hypertensive subjects (*10*–*14*).

Azelnidipine is a new and long-acting dihydropyridine calcium channel blocker. This drug has been shown to decrease blood pressure with a similar potency as other dihydropyridines, such as amlodipine, but without increasing pulse rate (*15*). The reason for azelnidipine's lack of stimulating action on the pulse rate has not been determined, but it is possible that azelnidipine may not stimulate the sympathetic nervous system, due to the slow appearance of its depressor effect. However, little is known about whether azelnidipine has a direct action on the sympathetic nervous system.

In the present study, we examined the effects of oral or intravenous administration of azelnidipine on the cardiovascular and sympathetic nervous system in conscious rats. Attention was focused on the action of azelnidipine in response to air-jet stress in rats that were awake. The results of azelnidipine were compared with those of nicardipine, another dihydropyridine calcium channel blocker.

Methods

Experimental Animals

Male stroke-prone spontaneously hypertensive rats (SHRSP; 10-week-old) were purchased from Japan SLC Inc. (Shizuoka, Japan), and fed standard laboratory rat chow and tap water ad libitum. The rats were kept in a room maintained at constant temperature and humidity under a 12-h light period between 8:00 AM and 8:00 PM. After adaptation to these conditions over at least 2 weeks, rats were divided into experimental groups, and the experimental procedures of each protocol were performed. All procedures were performed according to the National Institutes of Health guidelines for the care and use of laboratory animals. This experiment was approved by the Animal Care and Use Committee of the University of the Ryukyus.

Implantation of Arterial and Venous Catheters, and the Renal Nerve Electrode

Rats were anesthetized with an intraperitoneal injection of 50 mg/kg of sodium pentobarbital, and vascular catheters (PE-10 fused with PE-50) were inserted through the right femoral artery and vein for arterial pressure recording and drug administration, respectively. The left renal nerves were exposed through a retroperitoneal approach. A branch of the nerves was separated from the surrounding connective tissue, and a bipolar silver wire electrode (no.7855; A-M Systems, Carlsborg, USA) was placed under the nerve branch. When an optimal neurogram was obtained, the nerve and electrode were embedded in silicone gel (Semicosil 932; Wacker, Munich, Germany) and allowed to harden. Catheters and lead wires from the electrode were exteriorized at the interscapular region through a subcutaneous tunnel and fixed to the skin, as described previously (*16*, *17*).

Table 1. Basal MAP, HR and BW of Control, Low-Dose and High-Dose Azelnidipine, and Nicardipine Groups

Treatment group	MAP (mmHg)	HR (bpm)	BW (g)
Control	169±6	327±11	303±6
Azelnidipine 3 mg	157±3	333±6	294±8
Azelnidipine 10 mg	145±3*	334±10	287±8
Nicardipine	147±5*	333±8	290±5

Values are means±SEM, $n=6$ per group. MAP, mean arterial pressure; HR, heart rate; BW, body weight; bpm, beats per minute. *$p<0.05$ *vs*. control.

Recording Procedures

The experimental recording was performed with rats in a conscious and unrestrained state. Rats were allowed at least 18 h after surgery for recovery, which was considered adequate for resumption of regular eating, drinking and grooming habits, and then all experimental protocols were started. Each rat was placed in a plastic bowl with a diameter of 18 cm and a depth of 14 cm, and was allowed to move freely. During the recording period, acoustic disturbances were avoided, and the room was kept at constant temperature with a moderate degree of illumination.

All signals were digitized and recorded using a PowerLab system (PowerLab/4sp; ADInstruments, Tokyo, Japan) and used for further analysis. Arterial pressure was measured through a pressure transducer (P10EZ; Spectramed, Tokyo, Japan) and heart rate (HR) was monitored using a HR counter triggered by an arterial pressure pulse. Renal nerve signals were amplified by a biophysical amplifier (DPA-100E; Dia Medical System, Tokyo, Japan), and then filtered between 100 and 1,000 Hz; lastly, the signal was integrated by a PowerLab system with 1-s resetting. The integrated renal sympathetic nerve activity (RSNA) after intravenous administration of hexamethonium (40 mg/kg) was determined as the background noise level. Changes in RSNA were expressed as percent changes from baseline nerve activity.

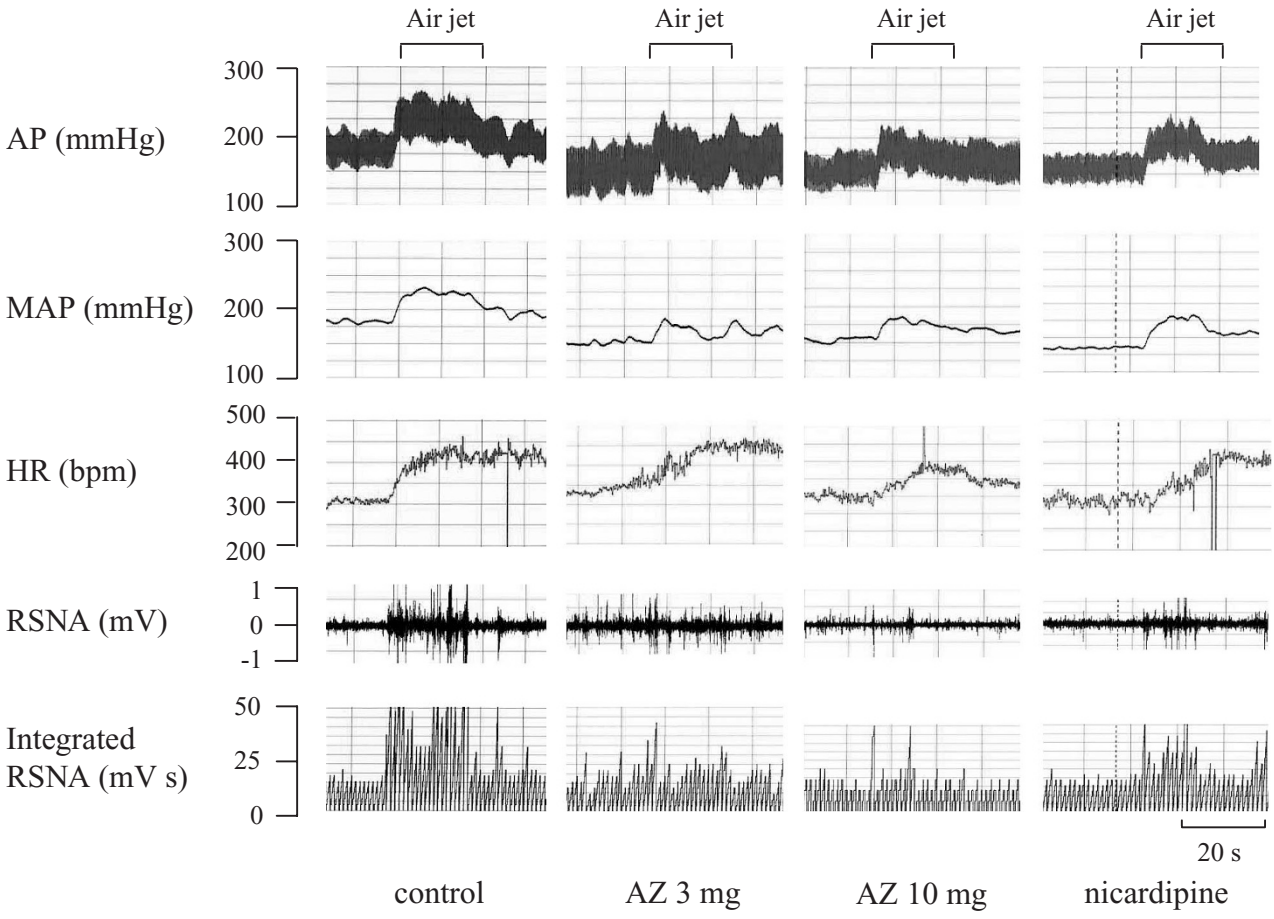

Fig. 1. *Typical recordings of the pulsatile arterial pressure (AP), mean arterial pressure (MAP), heart rate (HR), renal sympathetic nerve activity (RSNA), and integrated RSNA in response to air-jet stress in orally treated low-dose azelnidipine (3 mg/kg/day, AZ 3 mg), high-dose azelnidipine (10 mg/kg/day, AZ 10 mg), nicardipine (150 mg/kg/day), and control rats.*

Experimental Protocols

Response to Air-Jet Stress after Oral Administration of Azelnidipine or Nicardipine

SHRSP (12-week-old) were assigned to 4 groups (n=6 per group): a control group, low-azelnidipine group (3 mg/kg per day), high-azelnidipine group (10 mg/kg per day) and nicardipine group (150 mg/kg per day). All groups received standard rat chow. Drugs at each concentration were administered orally to the respective groups by mixing with standard chow for 10 days. At day 11, the operation was performed. The next day of the operation, baseline mean arterial pressure (MAP), HR and RSNA were recorded for rats in an unrestricted, awake condition, and then continuous air jets were administered for 20 s. An air jet, delivered from 8–10 cm apart, was directed at the face of the rat; the strength of the stimulus was adjusted so that it did not provoke increases in gross locomotive activity, as described previously (*18*). Changes in MAP, HR and integrated RSNA were evaluated.

Effects of Acute Intravenous Administration of Azelnidipine or Nicardipine on Cardiovascular and Sympathetic Nervous Systems in Response to Air-Jet Stress

SHRSP were assigned to 3 groups (n=6 per group): a control group, an azelnidipine group and a nicardipine group. After a stabilization period of at least 30 min, baseline MAP, HR and RSNA were recorded, and then azelnidipine (300 μg/kg, bolus for 1 min) or nicardipine (1–5 μg/kg/min, continuously by infusion pump) was administrated intravenously. The rate of infusion of nicardipine was adjusted such that the MAP was lowered over a time course and the degree of hypotension was similar to that during azelnidipine administration. MAP, HR and RSNA were continuously recorded for 60 min after administration of each drug; MAP, HR and integrated RSNA values were evaluated every 5 min for the first 15 min, and then every 15 min until 60 min after the drug administration. To investigate the effects of the air jet, continuous air-jet stress was induced for 20 s, 15 min after the administration of each drug. Changes in MAP, HR and integrated RSNA were evaluated.

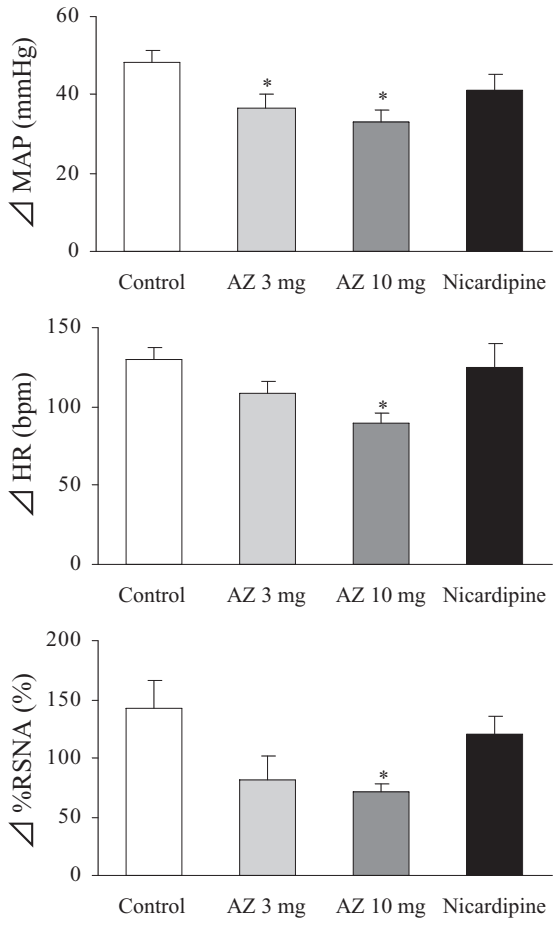

Fig. 2. *Responses to air-jet stress with or without oral treatment of azelnidipine (AZ) or nicardipine. Peak changes in MAP, HR and RSNA responses to air-jet stress are shown.* *p < *0.05* vs. *control rats. Data represent the means ± SEM,* n = *6 per group.*

Statistical Analysis

Values are expressed as the means ± SEM. Differences among the groups were tested by two-way analysis of variance with or without repeated measures. Subsequent analysis for significant difference was performed using Dunnet's or Sheffe's *F* test. All analyses were performed with the use of Statistical Analysis System (SAS) software (version 8.2; SAS Institute Inc., Cary, USA). A value of $p<0.05$ was considered significant.

Results

Air-Jet Stress: Effects of Oral Administration of Azelnidipine or Nicardipine

Rats treated or not treated with azelnidipine or nicardipine for 10 days were used. The baseline values for MAP and HR of the control, high-azelnidipine, and low-azelnidipine groups are shown in Table 1. In the high-azelnidipine and the nicardipine groups the baseline MAP was significantly lower than in the control group, but no significant decrease was observed in the low-azelnidipine group. Baseline HR was comparable among the four groups. Air-jet stress evoked increases in MAP, HR, and RSNA in all groups. Oral administration of either high-dose or low-dose azelnidipine significantly inhibited the pressor response to air-jet stress, whereas nicardipine did not (Figs. 1 and 2). Administration of azelnidipine at both high and low doses suppressed the HR and RSNA responses to air-jet stress, but statistical significance was observed only for the high dose.

Time-Dependent Change: Effects of Intravenous Administration of Azelnidipine or Nicardipine

A single injection of azelnidipine caused a slowly developing depressor response. The peak depressor response appeared at approximately 10 min after the injection, and the depressor response continued up to 60 min after the injection (Fig. 3A). Since the single injection of nicardipine caused a rapid decrease in MAP (data not shown), nicardipine was continuously infused at adjusted doses so as to obtain a time course of depressor response similar to that observed for azelnidipine. The time courses of the depressor response with azelnidipine and nicardipine overlapped, as we intended (Fig. 3B). During the depressor responses, HR and RSNA increased both in the azelnidipine and nicardipine groups (Fig. 3A). Changes in HR in the azelnidipine and nicardipine groups were comparable, but the increase in RSNA was smaller in the azelnidipine group than in the nicardipine group (Fig. 3B).

Air-Jet Stress: Effects of Intravenous Administration of Azelnidipine or Nicardipine

Finally, we investigated whether the cardiovascular and sympathetic responses to air-jet stress differed between azelnidipine and nicardipine when applied intravenously (Fig. 4). Fifteen minutes after the start of intravenous application of azelnidipine or nicardipine, rats were subjected to air-jet stress. Pretreatment with either azelnidipine or nicardipine inhibited the MAP response to air-jet stress, but the inhibition with azelnidipine was greater than that with nicardipine. The increase in HR in response to air-jet stress was significantly suppressed in both the azelnidipine and nicardipine groups compared with the control. The increase in RSNA in response to air-jet stress was significantly suppressed only in the azelnidipine group.

Discussion

The present study demonstrated that oral administration of azelnidipine attenuated the cardiovascular and sympathetic response to air-jet stress in conscious, unrestrained geneti-

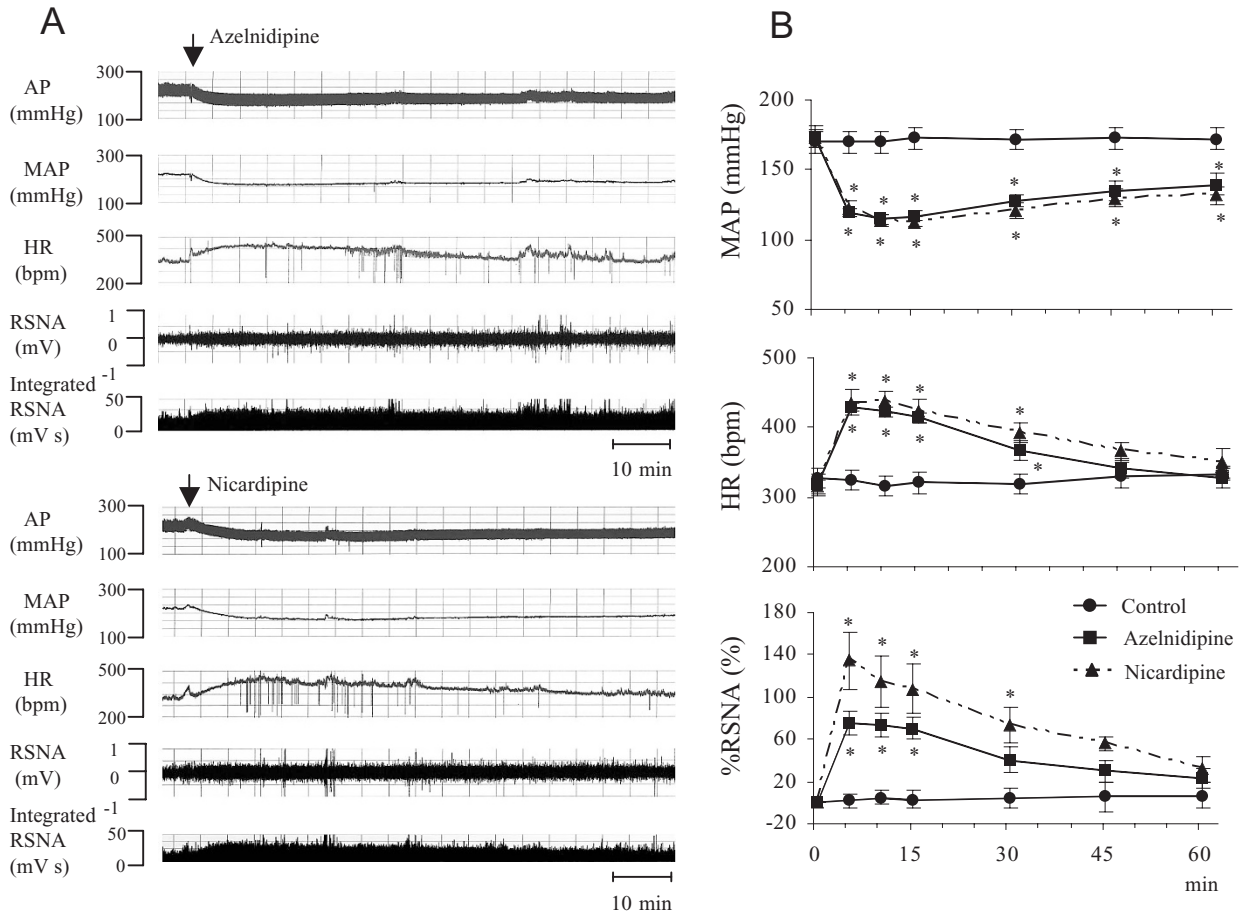

Fig. 3. *Time-dependent changes in MAP, HR, and RSNA after intravenous administration of azelnidipine or nicardipine. A: Typical recordings of AP, MAP, HR, and RSNA taken up to 60 min after administration of azelnidipine (300 μg/kg, i.v., bolus, for 1 min) or nicardipine (1–5 μg/kg/min, continuously by infusion pump). The arrows indicate the start of drug administration. Note that natural behavior causes the traces to fluctuate, but these changes were transient. B: Changes of MAP, HR and RSNA after intravenous administration of azelnidipine or nicardipine.* *$p < 0.05$ vs. *control rats. Data represent the means ± SEM,* $n = 6$ *per group.*

cally hypertensive rats. In contrast, oral administration of nicardipine did not significantly inhibit these stress-induced responses, despite having a depressor effect similar to that of azelnidipine. Since inhibition of stress-induced responses was observed with a non-depressor dose of azelnidipine, this inhibition could not have resulted from a decrease in the basal blood pressure level. Hosono *et al*. also reported that oral administration of classical dihydropyridines such as nifedipine, nicardipine or manidipine lowered blood pressure to a similar extent, but did not affect the pressor response to cold stress (*19*). These results together with our observations suggest that azelnidipine, when administered orally, could exert more potent inhibitory action on stress-induced responses than other classical dihydropyridines.

We also demonstrated that when administered intravenously, azelnidipine inhibited increases in MAP, HR and RSNA caused by air-jet stress. Nicardipine caused smaller inhibition of these parameters than azelnidipine. In a study using the same protocol, Takishita *et al*. reported that intravenously administered manidipine inhibited change in MAP, but not that in RSNA (*18*). Although most dihydropyridine drugs seem to have an effect on stress-induced responses, the potency of azelnidipine, when administered intravenously, appears to be relatively high among this group of drugs.

Various areas in the central nervous system, including the hypothalamus and brain stem, are involved in pressor and sympathoexcitatory responses to emotional or environmental stress (*20*). Among them, the rostral ventrolateral medulla (RVLM) in the brain stem has an important role in the regulation of sympathetic activity. The angiotensin type 1 (AT_1) receptor, production of reactive oxygen species (ROS) *via* NAD(P)H oxidase, and production of nitric oxide *via* nitric oxide synthase in RVLM have all been suggested to contribute significantly to the regulation of stress-induced sympa-

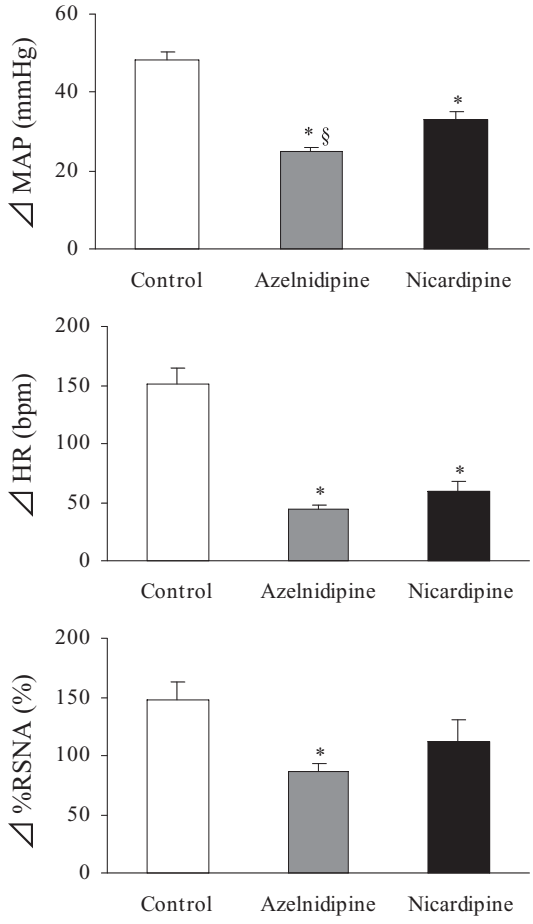

Fig. 4. *Responses of MAP, HR and RSNA to air-jet stress after intravenous administration of azelnidipine (300 μg/kg, i.v., bolus, 1 min) or nicardipine (1–5 μg/kg/min, continuously by infusion pump). Peak changes in response to air-jet stress are shown.* *$p < 0.05$ vs. *control rats.* §$p < 0.05$ vs. *nicardipine rats. Data represent the means ± SEM,* $n = 6$ *per group.*

thetic activation (*21–26*). Azelnidipine has been reported to suppress the production of ROS (*27–29*). If azelnidipine suppressed the production of ROS in the brain stem as well, this drug could inhibit the central sympathetic outflow.

Leenen *et al*. showed that intracerebroventricular administration of dihydropyridines such as nifedipine or amlodipine caused decreases in blood pressure, HR and RSNA in spontaneously hypertensive rats (SHR) (*30–32*). Both nifedipine and amlodipine, when administered intravenously at low rates for a long period, also decreased blood pressure, HR and RSNA, whereas a rapid injection increased HR and RSNA in response to a rapid decrease in blood pressure. These studies raise the hypothesis that orally or intravenously administered dihydropyridines may cross the blood-brain barrier and act on the central nervous system. Although no study is available to demonstrate whether azelnidipine crosses the blood-brain barrier, the inhibitory action of azelnidipine in the present study may be explained by its action on the central nervous system.

We showed that the activation of RSNA induced by an intravenous application of azelnidipine was smaller than that induced by nicardipine, whereas the HR responses induced by the two drugs were comparable. Shokoji *et al*. demonstrated that continuous infusion of azelnidipine exerted sympathoinhibitory effects on HR and RSNA through a reduction in baroreflex functions (*33*). It is therefore likely that the inhibitory action of azelnidipine on the sympathetic nervous system, including the baroreflex function system, may also explain our results, at least in part. Although we do not know why there is a discrepancy between the RSNA response and HR response in our results, it is possible that the inhibitory action on the sympathetic response, and especially on the HR response, did not reach a steady level in the first several minutes after intravenous administration of azelnidipine. Alternatively, azelnidipine may affect the vagal nervous activity or may differentially affect renal and cardiac sympathetic nerves.

It remains unknown how azelnidipine is different from nicardipine as regards the inhibition of the sympathetic nervous system. The mechanism may lie in the pharmacological differences between azelnidipine and nicardipine. For example, azelnidipine has greater lipophilicity than nicardipine. Lipophilic drugs tend to cross the blood-brain barrier, and may act on the brain. Another possible mechanism is that azelnidipine may act on non–L-type calcium (Ca) channels which distribute in neurons. For example, an oral administration of cilnidipine, which inhibits both N-type Ca channels and L-type Ca channels, has been shown to inhibit stress-induced increases in blood pressure in rats and humans (*19*, *34*). However, this possibility has not been evaluated yet in azelnidipine.

In the present study, oral administration of neither azelnidipine nor nicardipine affected basal HR. The absence of an increase in HR despite the significant reduction in blood pressure might result from the inhibitory action of both drugs on the basal sympathetic activity in the heart. Alternatively, the mechanism in the sympathetic nervous system that regulates basal HR and stress-induced responses might be different.

In summary, orally or intravenously administered azelnidipine attenuated the cardiovascular and sympathetic responses to air-jet stress. Azelnidipine also attenuated the reflex activation of the sympathetic nervous system induced by a decrease in blood pressure. These results suggest that azelnidipine may act on the sympathetic nervous system. Although the mechanism of this action remains unknown, this inhibition of the sympathetic response may benefit the treatment of hypertension, since exaggerated sympathetic activity is considered a risk factor for cardiovascular diseases.

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